

## CLAIMS

That which is claimed is:

- 5    1.    A method for detecting a target pathogen in a sample, the method comprising:
  - a)    providing a system comprising:
    - a layer of immobilized metal particles positioned on a surface substrate,
    - wherein the immobilized metal particles have attached thereto a captured
    - 10    biomolecular probe with an affinity for the target pathogen; and
    - a free biomolecular probe with an affinity for the target pathogen, wherein
    - the free biomolecular probe has attached thereto a fluorophore;
  - b)    contacting the sample with the immobilized biomolecular probes, wherein
  - the target pathogen binds to the immobilized biomolecular probes; and
  - 15    c)    contacting the bound target pathogen with the free biomolecular probe,
  - wherein binding of the free biomolecular probe to the target pathogen causes the
  - fluorophore to be positioned a sufficient distance from the immobilized metal
  - particles to enhance fluorescence emission when excited by an irradiating source.
- 20    2.    The method according to claim 1 wherein the immobilized and free biomolecular
- probes comprise a DNA sequence complementary to a target pathogen DNA sequence.
3.    The method according to claim 2, wherein the target pathogen is *B. anthracis*.
- 25    4.    The method according to claim 1, wherein the fluorophore is positioned from about
- 50 to about 500    from the immobilized metal particles after the free biomolecular probe
- contacts the target pathogen.
5.    The method according to claim 1, wherein the metal particles is silver or gold.

6. The method according to claim 1, further comprising detecting fluorescence emission with a detection device.
- 5 7. The method according to claim 6, wherein the detection device comprises a spectrometer, luminometer microscope, plate reader, fluorescent scanner, flow cytometer, or any combination thereof.
8. The method according to claim 4, wherein the immobilized biomolecular probe is  
10 covalently linked to the immobilized metallized particles.
9. The method according to claim 2, wherein binding of the immobilized and free DNA sequence complementary to the target pathogen DNA is conducted under high stringent hybridization conditions.
- 15 10. The method according to claim 1, wherein the irradiating source uses a 1-photon or 2-photon excitation means.
11. The method according to claim 1, wherein the biomolecular probe is an antibody  
20 and the target pathogen is an antigen.
12. The method according to claim 1, wherein the fluorophore comprises a low quantum yield species.
- 25 13. The method according to claim 1, wherein the fluorophore can undergo two-photon excitation.
14. The method according to claim 1, wherein the fluorophore comprises Rhodamine B, rose bengal or fluorescein isothiocyanate.

15. The method according to claim 1, wherein the free biomolecular probe further comprises a metal colloid attached thereto and positioned for sandwiching the fluorophore between the metal colloid and immobilized metal particles on the substrate when the target  
5 pathogen is bound.

16. An assay method for detecting a target pathogen in a sample, the method comprising:

10 a) providing a system comprising:

an immobilized metallized layer positioned on a surface substrate, wherein the immobilized metallized layer has attached thereto an immobilized capture DNA sequence probe complementary to a known DNA sequence of the target pathogen;  
and

15 a free capture DNA sequence probe complementary to a known DNA sequence of the target pathogen, wherein the free capture DNA sequence probe has attached thereto a fluorophore;

b) contacting the sample with the immobilized capture DNA sequence probe, wherein the DNA sequence of the target pathogen binds to the immobilized capture DNA  
20 sequence probe;

c) contacting the bound DNA sequence of the target pathogen with the free capture DNA sequence probe, wherein binding of the free capture DNA sequence probe to the DNA sequence of the target pathogen causes the fluorophore to be positioned a sufficient distance from the immobilized metallized surface to enhance fluorescence  
25 emission when excited by an irradiating source; and

d) identifying the target pathogen by fluorescence emission by irradiating the system with an irradiating source to excite the fluorophore.

17. The method according to claim 16, wherein the free capture DNA sequence probe further comprises a metal colloid attached thereto and positioned for sandwiching the fluorophore between the metal colloid and immobilized metal particles on the surface substrate when the DNA sequence of the target pathogen is bound to the immobilized metal particles.
18. The method according to claim 16, wherein the DNA sequence target pathogen is *B. anthracis*.
19. The method according to claim 16, wherein the fluorophore is positioned from about 50 to about 500 from the immobilized metallized surface after the free capture DNA sequence probe contacts the DNA sequence of the target pathogen.
20. The method according to claim 16, wherein the metallized surface comprises metal particles comprising silver or gold.
21. The method according to claim 16, further comprising detecting fluorescence emission with a detection device.
22. The method according to claim 21, wherein the detection device comprises a spectrometer, luminometer microscope, plate reader, fluorescent scanner, flow cytometer, or any combination thereof.
23. The method according to claim 16, wherein binding of the immobilized and free capture DNA sequence complementary to the target pathogen DNA is conducted under high stringent hybridization conditions.
24. The method according to claim 16, wherein the irradiating source uses a 1-photon or 2-photon excitation means.

25. The method according to claim 16, wherein the fluorophore comprises a low quantum yield species.

5 26. The method according to claim 16, wherein the fluorophore can undergo two-photon excitation.

27. The method according to claim 16, wherein the fluorophore comprises Rhodamine B, rose bengal or fluorescein isothiocyanate.

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28. An assay system for detecting a target pathogen in a sample, the system comprising:

a layer of immobilized metal particles deposited on a surface substrate, wherein a captured DNA probe complementary to a known DNA sequence of the target pathogen is  
15 immobilized on the metal particles;

a free DNA probe complementary to the known DNA of the target pathogen, wherein the free DNA probe has attached thereto a fluorophore; wherein binding of the immobilized and free DNA probe to the DNA sequence of the target pathogen causes the fluorophore to be positioned a sufficient distance from the immobilized metal particles to  
20 enhance fluorescence emission.

29. The system according to claim 28, wherein the free DNA sequence probe further comprises a metal colloid attached thereto and positioned for sandwiching the fluorophore between the metal colloid and immobilized metal particles on the surface substrate.

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30. The system according to claim 28, wherein the DNA sequence target pathogen is *B. anthracis*.

31. The system according to claim 28, wherein the metal particles is silver or gold.

32. The system according to claim 28, further comprising a detection device for detecting fluorescence emission.

5 33. The system method according to claim 32, wherein the detection device comprises a spectrometer, luminometer microscope, plate reader, fluorescent scanner, flow cytometer, or any combination thereof.

10 34. The system according to claim 28, wherein the fluorophore comprises a low quantum yield species.

36. The method according to claim 28, wherein the fluorophore can undergo two-photon excitation.

15 37. The method according to claim 28, wherein the fluorophore comprises Rhodamine B, rose bengal or fluorescein isothiocyanate.

38. An assay system for detecting a target pathogen comprising:

20 a layer of immobilized metal particles deposited on a surface substrate, wherein a captured biomolecular probe having an affinity for a target pathogen is immobilized on the metal particles;

a free biomolecular probe having an affinity for a target pathogen, wherein the free biomolecular probe has attached thereto a fluorophore; wherein binding of the immobilized and free biomolecular probe to the target pathogen causes the fluorophore to be positioned  
25 a sufficient distance from the immobilized metal particles to enhance fluorescence emission.

39. The system according to claim 38, wherein the immobilized and free biomolecular probes comprise a DNA sequence complementary to a target pathogen DNA sequence.

40. The system according to claim 38, wherein the target pathogen is *B. anthracis*.

41. The system according to claim 38, wherein the metal particles is silver or gold.

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42. The system according to claim 38, further comprising a detection device for detecting fluorescence emission.

43. The system according to claim 42, wherein the detection device comprises a spectrometer, luminometer microscope, plate reader, fluorescent scanner, flow cytometer, or any combination thereof.

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44. The system according to claim 38 further comprising an irradiating source.

15 45. The system according to claim 38, wherein the biomolecular probe is an antibody and the target pathogen is an antigen.

46. The system according to claim 38, wherein the fluorophore comprises a low quantum yield species.

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47. The system according to claim 38, wherein the fluorophore can undergo two-photon excitation.

48. The system according to claim 38, wherein the fluorophore comprises Rhodamine B, rose bengal or fluorescein isothiocyanate.

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49. The system according to claim 38, wherein the free biomolecular probe further comprises a metal colloid attached thereto and positioned for sandwiching the fluorophore between the metal colloid and immobilized metal particles on the substrate.

50. A kit for detecting a target pathogen in a sample, the kit comprising  
a container comprising a layer of immobilized metal particles deposited on a  
surface substrate, wherein a captured biomolecular probe is immobilized on the metal  
5 particles and wherein the captured biomolecular probe has an affinity for a target pathogen  
of interest ; and

a free biomolecular probe having an affinity for a target pathogen, wherein the free  
biomolecular probe has attached thereto a fluorophore and wherein binding of the target  
pathogen to both the immobilized and free biomolecular probe causes the fluorophore to be  
10 positioned a sufficient distance from the immobilized metal particles to enhance  
fluorescence emission, wherein the immobilized and free biomolecular probe can be in the  
same or different containers.

51. The kit according to claim 50, wherein the immobilized and free biomolecular  
15 probes comprise a DNA sequence complementary to a target pathogen DNA sequence.

52. The kit according to claim 51, wherein the target pathogen is *B. anthracis*.

53. The kit according to claim 50, wherein the metal particles is silver or gold.  
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54. The kit according to claim 50, wherein the fluorophore comprises a low quantum  
yield species.

55. The kit according to claim 50, wherein the fluorophore comprises Rhodamine B,  
25 rose bengal or fluorescein isothiocyanate.

56. The kit according to claim 50, wherein the free biomolecular probe further  
comprises a metal colloid attached thereto and positioned for sandwiching the fluorophore  
between the metal colloid and immobilized metal particles on the substrate.